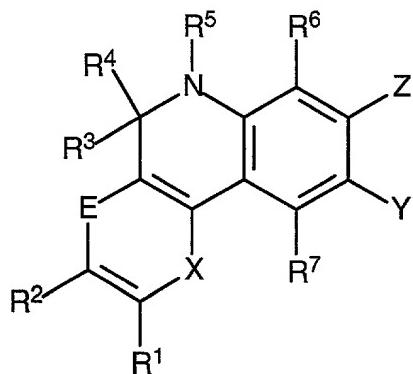


We claim

1. A compound of the formula

5



wherein

10 R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

15

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or which ring is substituted by -L-R<sub>x</sub> or -L-S<sub>c</sub>;

or R<sup>2</sup> in combination with R<sup>3</sup> forms a 5- or 6-membered alicyclic ring;

20 R<sup>3</sup> and R<sup>4</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

25 or R<sup>3</sup> in combination with R<sup>4</sup> forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen,

5 carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

R<sup>6</sup> is H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl,

10 cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

15 R<sup>7</sup> is hydrogen, alkyl having 1-6 carbons, or alkoxy having 1-6 carbons; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>, and the other is absent;

20 wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>:  
and

25 R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

Y is H, OH, NH<sub>2</sub>, NO, or -(CO)-R<sup>9</sup>, or -(CO)-O-R<sup>10</sup>, where R<sup>9</sup> and R<sup>10</sup> are H, C<sub>1</sub>-C<sub>6</sub> alkyl, or a

30 substituted or unsubstituted aryl or heteroaryl ring system having 1-2 rings;

Z is H, OH, NHR<sup>17</sup>, SH, or C(CR<sup>11</sup>R<sup>12</sup>)<sub>2</sub>OH; where R<sup>17</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; R<sup>11</sup> and R<sup>12</sup> are independently C<sub>1</sub>-C<sub>6</sub> alkyls that are optionally substituted by carboxylic acid, sulfonic acid, or halogen, or R<sup>11</sup> and R<sup>12</sup> taken in combination form a 5- or 6-membered alicyclic ring;

5

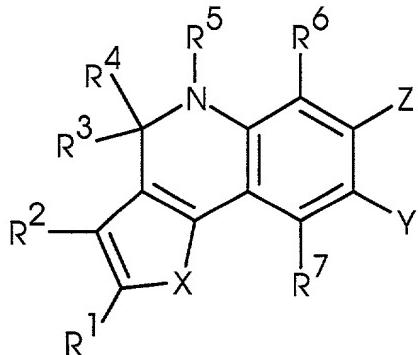
wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

10 S<sub>c</sub> is a conjugated substance.

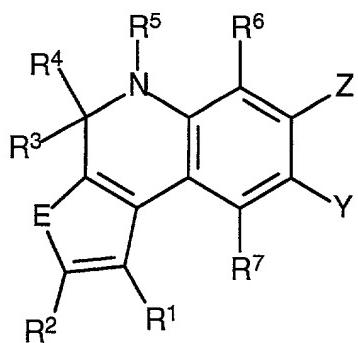
2. A compound, as claimed in Claim 1, wherein one of X and E is O, S, or CR<sup>1'</sup>=CR<sup>2'</sup>, and the other is absent.

15 3. A compound, as claimed in Claim 1, having the formula



wherein X is O or S.

20 4. A compound, as claimed in Claim 1, having the formula



wherein E is O or S.

5 5. A compound, as claimed in Claim 2, wherein X is S.

6. A compound as claimed in Claim 1, wherein

R<sup>1</sup> is H or sulfonic acid;

10 R<sup>3</sup> and R<sup>4</sup> are each methyl;

15 R<sup>6</sup> and R<sup>7</sup> are each hydrogen or methyl; and

Z is OH.

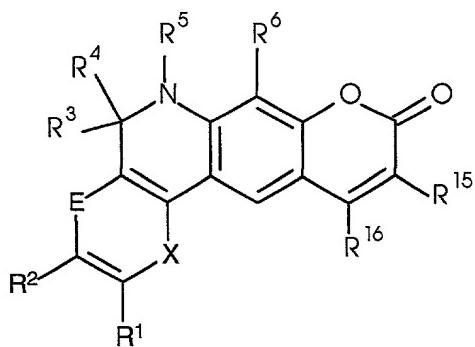
7. A compound, as claimed in Claim 1, wherein Y is H or -(CO)-H or NO.

8. A compound, as claimed in Claim 1, wherein each L is independently a single covalent  
20 bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group  
consisting of C, N, O, P, and S and is composed of any combination of single, double, triple  
or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds,  
carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-  
nitrogen bonds.

9. A compound, as claimed in Claim 1, wherein  $R_x$  is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an  
5 isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

10. A compound, as claimed in Claim 1, wherein  $S_c$  is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a  
10 nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

11. A compound of the formula



- 5    R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;
- 10    or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;
- 15    R<sup>3</sup> and R<sup>4</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;
- 20    or R<sup>2</sup> in combination with R<sup>3</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, forms a 5- or 6-membered alicyclic ring;
- 25    R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

5 one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>; and

10

R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

15

R<sup>15</sup> and R<sup>16</sup> are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

20

wherein L is a covalent linkage;

25

R<sub>x</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

12. A compound, as claimed in Claim 11, wherein one of X and E is O or S.

30

13. A compound, as claimed in Claim 12, wherein

R<sup>8</sup> and R<sup>7</sup> are H;

R<sup>3</sup> and R<sup>4</sup> are each methyl;

5

R<sup>1</sup> is H or sulfonic acid;

one of R<sup>15</sup> and R<sup>16</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>, and the other is hydrogen, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl; or cyano;

10

wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus-nitrogen bonds, and

15

wherein R<sub>x</sub>, when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group; and

20

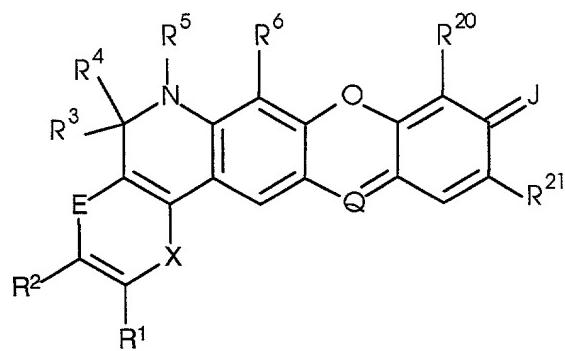
wherein S<sub>C</sub>, when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

25

14. A compound, as claimed in Claim 11, wherein one of R<sup>15</sup> and R<sup>16</sup> is an aromatic or heteroaromatic ring system having 1-2 fused rings that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl.

30

15. A compound of the formula



5 wherein

R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R<sup>3</sup> and R<sup>4</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>2</sup> in combination with R<sup>3</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally

substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or R<sup>5</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered  
5 alicyclic ring;

one of X and E is O, S, NR<sup>8</sup>, or CR<sup>1'</sup>=CR<sup>2'</sup>; and the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally  
10 substituted by carboxylic acid, sulfonic acid, amino, or halogen; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;  
and

R<sup>1'</sup> and R<sup>2'</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a  
C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid,  
15 or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more  
times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic  
acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

R<sup>20</sup> and R<sup>21</sup> are hydrogen, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or  
20 alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an  
aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub>  
alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or  
-L-R<sub>x</sub>; or -L-S<sub>c</sub>;

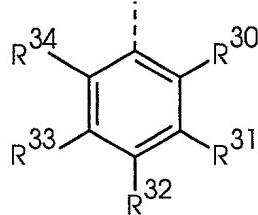
25 J is O or NR<sup>37</sup>R<sup>38</sup>;

where R<sup>37</sup> and R<sup>38</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by  
carboxylic acid, sulfonic acid, amino, or halogen; an aryl or heteroaryl ring; or R<sup>37</sup> in  
30 combination with R<sup>38</sup> forms a saturated 5- or 6-membered heterocycle that is a  
piperidine, a morpholine, a pyrrolidine or a piperazine, each of which is optionally  
substituted by methyl, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alkyl; or

-L-R<sub>x</sub> or -L-S<sub>c</sub>;

or R<sup>37</sup> in combination with R<sup>20</sup>, or R<sup>38</sup> in combination with R<sup>21</sup>, or both, form a 5- or 6-membered ring that is saturated or unsaturated, and is optionally substituted by  
5 one or more sulfonic acids, or C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by sulfonic acid;

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid,  
10 amino, or halogen; or R<sup>28</sup> has the formula



where R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, or C<sub>6</sub>-C<sub>18</sub> arylcarboxamido, the alkyl or aryl portions of which are  
15 optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, sulfonic acid, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents  
R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>; and

20 25 wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

**S<sub>c</sub>** is a conjugated substance.

16. A compound, as claimed in Claim 15, wherein Q is N.

5

17. A compound, as claimed in Claim 15, wherein J is O and Q is CR<sup>28</sup>.

18. A compound, as claimed in Claim 17, wherein one of R<sup>5</sup>, R<sup>21</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>.

10

19. A compound, as claimed in Claim 15, wherein

R<sup>3</sup> and R<sup>4</sup> are each methyl;

15

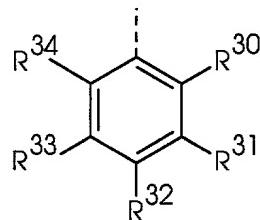
R<sup>1</sup> is H or a sulfonic acid;

R<sup>6</sup> is H; and

J is NR<sup>37</sup>R<sup>38</sup>.

20

20. A compound, as claimed in Claim 19, wherein Q has the formula CR<sup>28</sup>, wherein R<sup>28</sup> has the formula



25

wherein one of R<sup>30</sup>-R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>; and

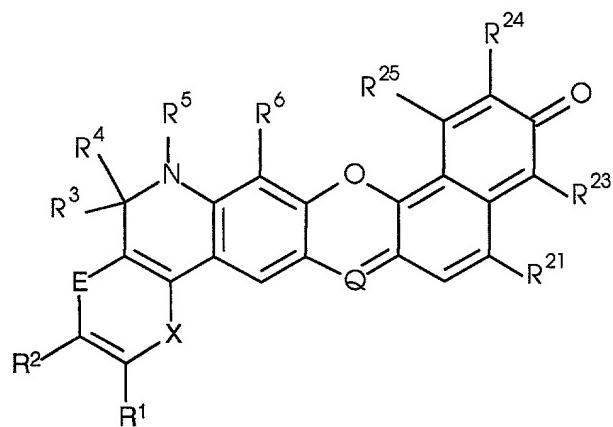
wherein L is a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen

atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus-nitrogen bonds, and

- 5 wherein R<sub>x</sub>, when present, is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl  
10 halide, or a thiol group; and  
wherein S<sub>c</sub>, when present, is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

15

21. A compound of the formula



R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>1</sub>; or -L-S<sub>C</sub>;

or R<sup>1</sup> in combination with R<sup>2</sup> forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R<sup>3</sup> and R<sup>4</sup> are independently C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>2</sup> in combination with R<sup>3</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> is H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

5

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, forms a 5- or 6-membered alicyclic ring;

one of E and X is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent;

10

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

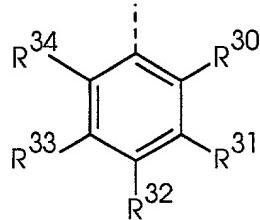
15 R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

20

R<sup>21</sup>, R<sup>23</sup>, R<sup>24</sup>, and R<sup>25</sup> are hydrogen, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

25

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> has the formula



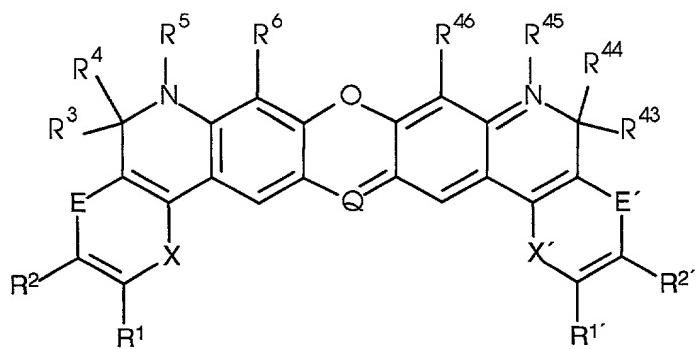
where R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, or C<sub>6</sub>-C<sub>18</sub> arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>C</sub>; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

22. A compound having the formula



5 wherein

R<sup>1</sup>, R<sup>2</sup>, R<sup>6</sup>, R<sup>41</sup>, R<sup>42</sup>, and R<sup>46</sup> are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>1</sup> in combination with R<sup>2</sup>, or R<sup>41</sup> in combination with R<sup>42</sup>, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R<sup>3</sup>, R<sup>4</sup>, R<sup>43</sup>, and R<sup>44</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>2</sup> in combination with R<sup>3</sup>, R<sup>42</sup> in combination with R<sup>43</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, or R<sup>43</sup> in combination with R<sup>44</sup>, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> and R<sup>45</sup> are independently H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

5

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, or R<sup>44</sup> in combination with R<sup>45</sup>, or R<sup>45</sup> in combination with R<sup>46</sup>, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

- 10 one of E and X is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent; and one of E' and X' is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent;

wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

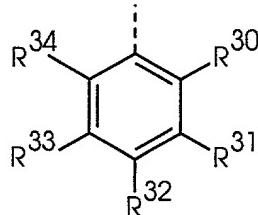
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R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

20

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> has the formula

25



where R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, or C<sub>6</sub>-C<sub>18</sub> arylcarboxamido, the alkyl or aryl portions of which are

- 5     optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup>  
10    is -L-R<sub>x</sub> or -L-S<sub>C</sub>; and

wherein L is a covalent linkage;

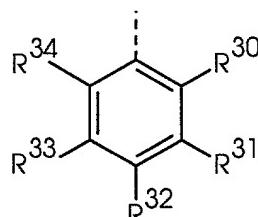
R<sub>x</sub> is a reactive group; and

S<sub>C</sub> is a conjugated substance.

23. A compound, as claimed in Claim 22, wherein

20    X = X', E = E', R<sup>1</sup> = R<sup>41</sup>, and R<sup>2</sup> = R<sup>42</sup>.

24. A compound, as claimed in Claim 22, wherein Q has the formula CR<sup>28</sup>, and R<sup>28</sup> has the formula



25. A compound, as claimed in Claim 24, wherein one of R<sup>5</sup>, R<sup>21</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, and R<sup>45</sup>  
is -L-R<sub>x</sub> or -L-S<sub>c</sub>.

26. A compound, as claimed in Claim 24, wherein

5

R<sup>3</sup>, R<sup>4</sup>, R<sup>43</sup>, and R<sup>44</sup> are each methyl;

R<sup>1</sup> and R<sup>41</sup> are independently H or sulfonic acid; and

10 R<sup>6</sup> and R<sup>46</sup> are H.

27. A compound, as claimed in Claim 24, wherein the compound is substituted one or more times by sulfonic acid.

15 28. A compound, as claimed in Claim 22, wherein one of R<sup>1</sup>, R<sup>1'</sup>, R<sup>2</sup>, R<sup>2'</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>,  
R<sup>15</sup>, R<sup>16</sup>, R<sup>20</sup>, R<sup>21</sup>, R<sup>23</sup>, R<sup>24</sup>, R<sup>25</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>37</sup>, R<sup>38</sup>, R<sup>41</sup>, R<sup>42</sup>, R<sup>43</sup>, R<sup>44</sup>, R<sup>45</sup>, and R<sup>46</sup> is an -L-  
-R<sub>x</sub> or -L-S<sub>c</sub>.

20 29. A compound, as claimed in Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon–carbon bonds, carbon–nitrogen bonds, nitrogen–nitrogen bonds, carbon–oxygen bonds, carbon–sulfur bonds, phosphorus–oxygen bonds, and phosphorus–nitrogen bonds.

25

30 30. A compound, as claimed in Claim 28, wherein R<sub>x</sub> is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, or a thiol group.

31. A compound, as claimed in Claim 28, wherein  $R_x$  is a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, or a reactive platinum

5 complex.

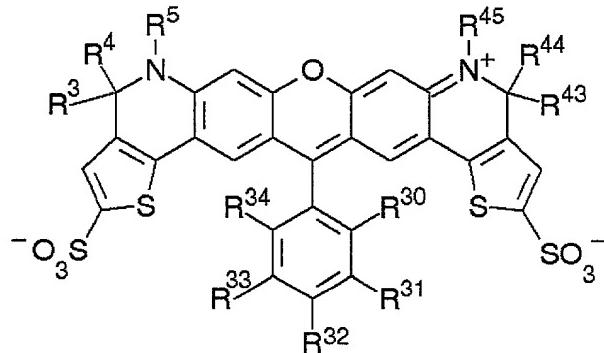
32. A compound, as claimed in Claim 28, wherein  $S_c$  is an amino acid, a peptide, a protein, a tyramine, a monosaccharide, a polysaccharide, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, or a virus.

10

33. A compound, as claimed in Claim 28, wherein  $S_c$  is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

15

34. A compound, as claimed in Claim 28, having the formula:



wherein  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^{43}$ ,  $R^{44}$ , and  $R^{45}$  are independently methyl or ethyl;

20  $R^{30}$  is sulfonic acid or carboxylic acid;

$R^{31}$  and  $R^{34}$  are independently H, F, or Cl;

one of  $R^{32}$  and  $R^{33}$  is H, F, or Cl, and the other of  $R^{32}$  and  $R^{33}$  is -L- $R_x$  or -L- $S_c$ ,

wherein L is a covalent linkage of the formula  $-S(CH_2)_aCOO(CH_2)_b-$  or the formula

$-S(CH_2)_aCONH(CH_2)_b-$

wherein a is an integer between 0 and 10, and b is an integer between 0 and 10  
provided that a and b are not both 0; and

wherein R<sub>x</sub>, where present, is a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, or a reactive platinum complex.; and

wherein S<sub>c</sub>, where present, is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid.

5

35. A compound, as claimed in Claim 34, wherein R<sub>x</sub> is a maleimide group or is a  
10 succinimidyl ester of a carboxylic acid.

36. A compound, as claimed in Claim 34, wherein S<sub>c</sub> is peptide or a protein or a lectin.

15

37. A compound, as claimed in Claim 34, wherein S<sub>c</sub> is an antibody or antibody fragment.

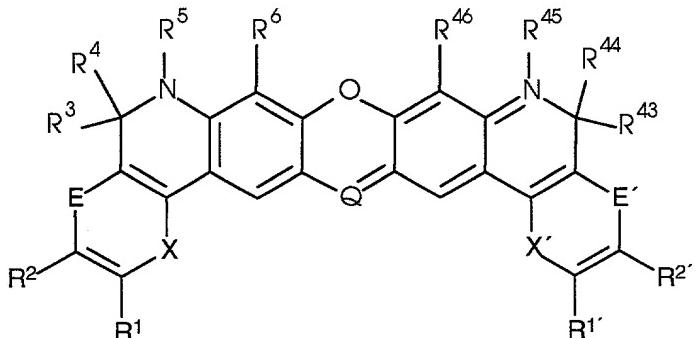
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38. A compound, as claimed in Claim 34, wherein S<sub>c</sub> is a nucleotide or an oligonucleotide.

20

39. A compound, as claimed in Claim 34, wherein S<sub>c</sub> is a BAPTA or APTRA ion-complexing moiety.

40. A method of staining a biological sample, comprising:  
combining a dye solution comprising a compound of the formula



5

wherein

R<sup>1</sup>, R<sup>2</sup>, R<sup>6</sup>, R<sup>41</sup>, R<sup>42</sup>, and R<sup>46</sup> are independently H, cyano, nitro, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl; or -L-R<sub>x</sub>; or -L-S<sub>C</sub>;

or R<sup>1</sup> in combination with R<sup>2</sup>, or R<sup>41</sup> in combination with R<sup>42</sup>, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times;

R<sup>3</sup>, R<sup>4</sup>, R<sup>43</sup>, and R<sup>44</sup> are independently H, C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen; or an aromatic or heteroaromatic ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>2</sup> in combination with R<sup>3</sup>, R<sup>42</sup> in combination with R<sup>43</sup>, or R<sup>3</sup> in combination with R<sup>4</sup>, or R<sup>43</sup> in combination with R<sup>44</sup>, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R<sup>5</sup> and R<sup>45</sup> are independently H, methyl, carboxymethyl, a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>5</sup> is an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub>

5 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R<sup>4</sup> in combination with R<sup>5</sup>, or R<sup>5</sup> in combination with R<sup>6</sup>, or R<sup>44</sup> in combination with R<sup>45</sup>, or R<sup>45</sup> in combination with R<sup>46</sup>, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

10

one of E and X is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent; and one of E' and X' is O, S, NR<sup>8</sup>, or CR<sup>1</sup>=CR<sup>2</sup>; the other is absent;

15

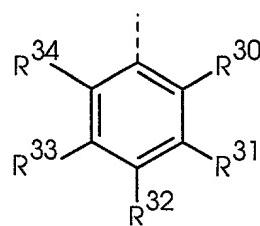
wherein R<sup>8</sup> is H, methyl, carboxymethyl, or a C<sub>2</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

20

R<sup>1</sup> and R<sup>2</sup> are independently H, cyano, halogen, carboxylic acid, or sulfonic acid; or a C<sub>1</sub>-C<sub>6</sub> alkyl or alkoxy that is optionally substituted by carboxylic acid, sulfonic acid, or halogen; or an aryl or heteroaryl ring that is optionally substituted one or more times by C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

25

Q is N or CR<sup>28</sup>, wherein R<sup>28</sup> is H, F, CN, carboxylic acid, or a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol; or R<sup>28</sup> is a C<sub>1</sub>-C<sub>6</sub> alkyl that is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R<sup>28</sup> has the formula



where R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> are independently H, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino; or C<sub>1</sub>-C<sub>18</sub> alkyl, C<sub>1</sub>-C<sub>18</sub> alkoxy, C<sub>1</sub>-C<sub>18</sub> alkylthio, C<sub>1</sub>-C<sub>18</sub> alkanoylamino, C<sub>1</sub>-C<sub>18</sub> alkylaminocarbonyl, C<sub>2</sub>-C<sub>36</sub> dialkylaminocarbonyl, C<sub>1</sub>-C<sub>18</sub> alkyloxycarbonyl, or C<sub>6</sub>-C<sub>18</sub> arylcarboxamido, the alkyl or aryl portions of which are optionally substituted one or more times by F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C<sub>1</sub>-C<sub>6</sub> alcohol, amino, alkylamino, dialkylamino or alkoxy, the alkyl portions of each having 1-6 carbons; or one pair of adjacent substituents R<sup>31</sup> and R<sup>32</sup>, R<sup>32</sup> and R<sup>33</sup> or R<sup>33</sup> and R<sup>34</sup>, when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; or one or more of R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup> and R<sup>34</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>; and

wherein L is a covalent linkage;

R<sub>x</sub> is a reactive group; and

S<sub>c</sub> is a conjugated substance;

with a biological sample in a concentration sufficient to yield a detectable optical response under the desired conditions.

41. A method, as claimed in Claim 40, further comprising combining the sample with an additional detection reagent that has spectral properties that are detectably different from said optical response.

42. A method, as claimed in Claim 40, further comprising the step of determining a characteristic of the sample by comparing the optical response with a standard response parameter.

43. A method, as claimed in Claim 40, wherein the sample comprises cells.

44. A method, as claimed in Claim 40, wherein the sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

5       45. A method, as claimed in Claim 40, further comprising tracing the temporal or spatial location of the optical response within the sample.

46. A method, as claimed in Claim 40, wherein for said compound

10           at least one of R<sup>28</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>37</sup> and R<sup>38</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>;

R<sub>x</sub> is a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, or a maleimide group; and

15           S<sub>c</sub> is an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, that is optionally bound to one or more additional fluorophores that are the same or different.

20           47. A method, as claimed in Claim 46, wherein for said compound, R<sup>28</sup> is an -L-S<sub>c</sub>, and S<sub>c</sub> is an ion-complexing moiety that is a BAPTA or an APTRA.

25           48. A method as claimed in Claim 40, wherein at least one of R<sup>28</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, R<sup>33</sup>, R<sup>34</sup>, R<sup>37</sup> and R<sup>38</sup> is -L-S<sub>c</sub>, and S<sub>c</sub> is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.